A Novel De Novo Frameshift Mutation in *KAT6A* Identified by Whole Exome Sequencing

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Abstract

Intellectual disability is a common condition with multiple etiologies. The number of monogenic causes has increased steadily in recent years due to the implementation of next generation sequencing. Here, we describe a 2-year-old boy with global developmental delay and intellectual disability. The child had feeding difficulties since birth. He had delayed motor skills and muscular hypotonia. Brain magnetic resonance imaging revealed diffuse white matter loss and thinning of the corpus callosum. Banded karyotype and comparative genomic hybridization (CGH) array were normal. Whole exome sequencing revealed a novel de novo frameshift mutation c.3390delA (p. Lys1130Asnfs*4) in *KAT6A* gene (NM_006766.4). The heterozygous mutation was confirmed by Sanger sequencing in the patient and its absence in his parents. *KAT6A* that encodes a histone acetyltransferase has been recently found to be associated with a neurodevelopmental disorder autosomal dominant mental retardation 32 (OMIM: no. 616268). Features of this disorder are nonspecific, which makes it difficult to characterize the condition based on the clinical symptoms alone. Therefore, our findings confirm the utility of whole exome sequencing to quickly and reliably identify the etiology of such conditions.

Keywords
- developmental delay
- intellectual disability
- exome
- KAT6A

Introduction

Neurodevelopmental disorders (NDDs) are a heterogeneous group of conditions generally identified early in life. NDDs can include intellectual disability, epilepsy, developmental delay, and autism spectrum disorders. Genetic etiology of NDDs is highly heterogeneous and novel genes/mutations are regularly being identified shedding light on finer classification of phenotypes and better understanding of the pathophysiology of disease. Currently, whole exome sequencing (WES) is a cost-effective tool of choice to identify causative genes in those patients with unclear phenotypic patterns.[1,2]

In this article, we describe a novel de novo mutation identified by clinical WES in lysine acetyltransferase 6A (*KAT6A* [OMIM 601408]), a gene recently identified in patients with global developmental delay and intellectual disability.[3,4] This novel mutation exemplifies the power of clinical WES and correlates the clinical phenotype of this patient with those reported previously.

Patients and Methods

Patient

We report a 2-year-old male patient, the fourth child from nonconsanguineous parents (►**Fig. 1**). The patient's three siblings are healthy. The patient was born at 40 weeks after an uneventful pregnancy and delivery, with normal ultrasounds and maternal serum tests. At time of the child's birth, the mother was 35 years old and the father 46 years old. The father reported conception occurred approximately 20 hours post exposure to an airport full-body security scanner. His weight at birth was 3.77 kg, length 53 cm, and head circumference

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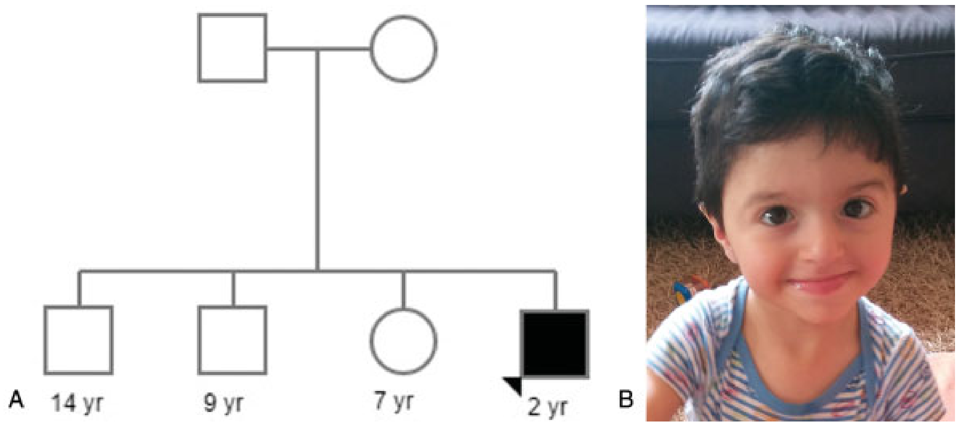

Fig. 1 (A) Family pedigree. The patient symbol is filled black and indicated with an arrow. (B) Facial features of the patient at 26 months of age. No major abnormal facial features are present.

35 cm. He had normal red reflex and absent congenital malformations. The child had feeding difficulties that required 4-day neonatal care unit admission with antibiotic administration as a precaution to perinatal infection. The patient had spastic neck muscles, delayed gross motor development, and no seizures. He was able to sit at 12 months, and at the time of manuscript submission (25 months of age) he was unable to stand and was nonverbal. From 6 months of age, he started physical therapy that slowly improved his motor skills and now he can grab objects and partially coordinate movement of both hands. Hearing evaluation was normal as was echocardiography. The patient was found to have a unilateral preauricular sinus, thin upper lip, and full lower lip. Amino acid analysis was negative. No similar phenotype was reported in the family.

Methods

Peripheral blood samples were collected from the patient and both parents. Informed consent was obtained and all procedures followed were in accordance with the ethical standards of the Jordan University of Science and Technology Institutional Review Board. DNA was extracted using QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to manufacturer's protocol. Chromosomal microarray was performed utilizing the Cytoscan750K platform (Affymetrix, Santa Clara, California, United States). The array has a density of 550k oligonucleotides and 200k single-nucleotide polymorphisms. Hybridization results were analyzed to detect genetic losses/gains, losses of heterozygosity, and uniparental disomy using ShAS 3.0 software (Chromosome Suite Analysis, Affymetrix).

Whole exome capture and sequencing were performed at Centogene Laboratory (Rostock, Germany). Approximately 60 MB of human exons (targeting $>$ 99% of regions in consensus coding sequence project, Gencode, and RefSeq databases) were enriched from 1 µg of fragmented genomic DNA using the SureSelect Human All Exon V6 Kit (Agilent, Santa Clara, California, United States) followed by processing on NextSeq platform (Illumina, San Diego, California, United States) to obtain an average coverage of $\sim$100X. Approximately 97% of targeted bases are covered $>$ 10X. An in-house bioinformatics pipeline was applied including base-pair calling, alignment of reads to genome assembly GRCh37/hg19, filtering out low-quality reads, and variants annotation. All disease-causing variants in CentoMD,[5] ClinVar,[6] and HMGD[7] were considered. Additionally, all variants in gnomAD database[8] with a minor allele frequency of $<$1% were considered. Evaluation of identified variants was focused on coding exons and their flanking intronic bases with consideration of multiple inheritance patterns. Further, clinical data and family history were considered for evaluating identified variants. Sanger sequencing was performed to confirm identified variants. Only variations in genes potentially related to the patient's clinical phenotype were reported. The *KAT6A* mutation in exon 17 was amplified and sequenced through standard procedures using the primers: forward-tgctagtcttggaagaaggaac and reverse-TCCTGGATCTTGGGTTTACG with an annealing temperature of 55 degrees and 35 amplification cycles.

Results

The child is shown in ►**Fig. 1** and as can be seen, he does not have any major abnormal morphological features.

MRI was conducted at 6 and 24 months of age. At 6 months, MRI showed white matter volume loss with thinning of the corpus callosum. There were no abnormal signal intensities in the white matter, no focal brain lesions, no mass effect of central midline structures, and no hemorrhage or other fluid collection. Further, the ventricular system appeared normal and myelination was appropriate for the patient's age. Basal ganglia, both thalami, brain stem, cerebellum, and paranasal sinuses appeared normal. At 24 months, mild thinning of the corpus callosum was again seen, with abnormal high intensity in the white matter of the terminal zones. In comparison to the 6-month MRI, there was an interval improvement in the volume of the white matter with progressive normal myelination that remined appropriate for patient's age. Thalami, basal ganglia, brain stem, cerebellum, and ventricular system appeared normal.

Multiple tests (including ammonia, kidney and liver function tests, amino acids, lactate, pyruvate, thyroid function tests, and ferritin) did not show deviation from normal ranges. These tests were done at 7 and 22 months of age. Array CGH showed a normal result with no gains or loss of genetic material. Clinical WES identified a heterozygous de novo nonsense mutation c.3390delA (NM_006766.4) in exon 17 of *KAT6A* gene (OMIM #601408) resulting in a frameshift, p. Lys1130Asnfs*4 (►**Fig. 2**). The mutation results in a stop codon 4 codons downstream of the mutation in the same exon. The coverage around the mutation was 123X. This mutation is novel, not reported in the 1000 genome project, the Exome Aggregation Consortium, nor the NHLPI Exome Sequencing project.

Discussion

Here, we describe the clinical case of a patient with non-specific features characterized by global developmental

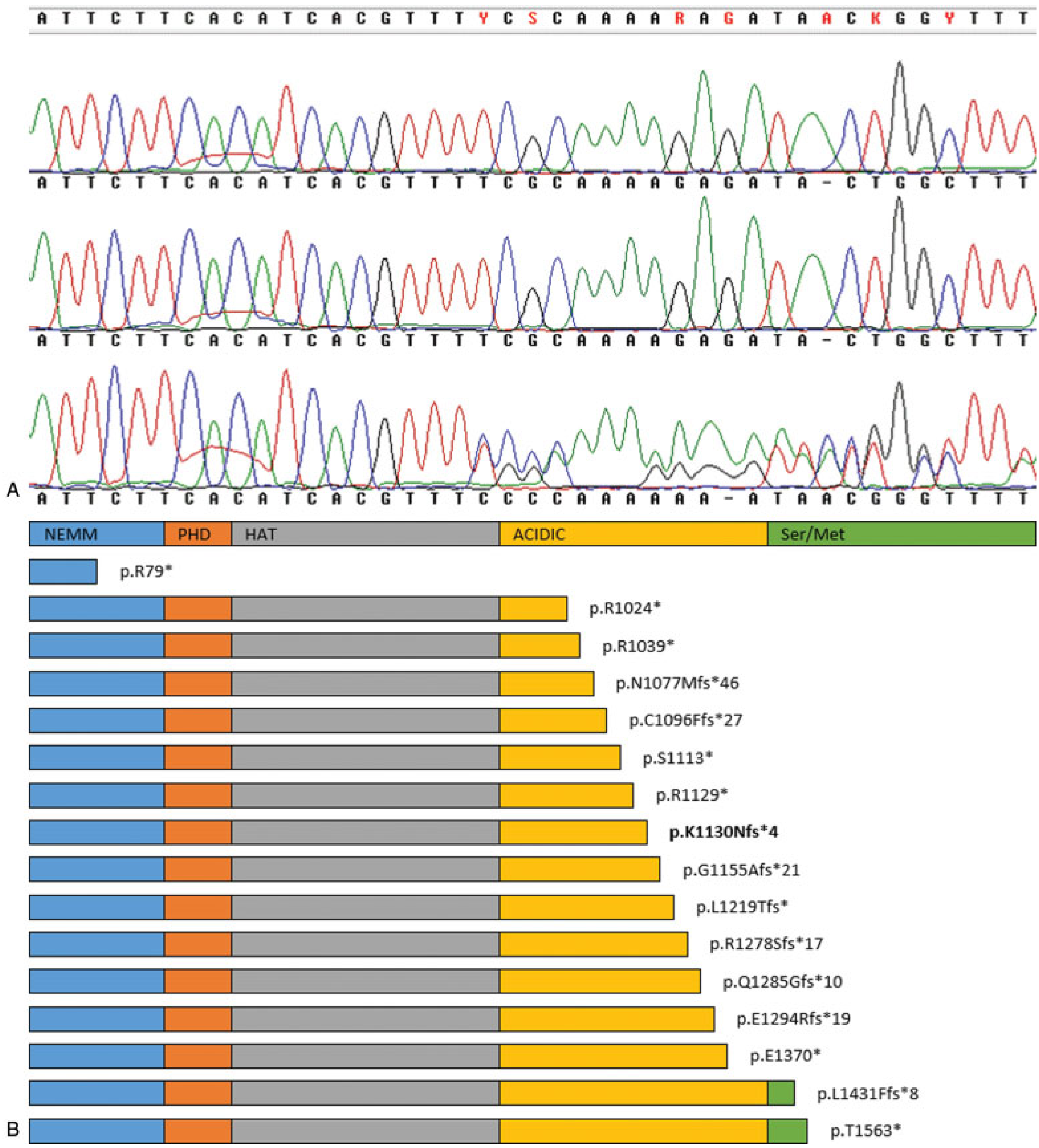

Fig. 2 (**A**) *KAT6A* Sanger sequencing traces for the patient (lower) and his parents (upper and middle), showing the single base-pair deletion (c.3390delA) that results in frameshift and consequent truncation of the protein 4 amino acids downstream (p.Lys1130Asnfs*4). Note that the sequence is present in the reverse orientation. (**B**) A schematic illustration of the different domains in the KAT6A protein; NEMM, PHD, HAT, ACIDIC, and SER/MET. The truncated KAT6A forms reported to date (frameshift and nonsense mutations, including our patient's in bold) are shown below. Note that the majority of truncating mutations are within the ACIDIC domain. ACIDIC, acidic glutamate/aspartate-rich domain; HAT, histone acetyltransferase domain; NEMM, nuclear localization domain; PHD, plant zinc finger homeodomain; SER/MET, serine and methionine-rich transactivation domain.

Table 1 A summary of *KAT6A* mutations reported to date

Mutation (nucleotide)	Mutation (protein)	Domain of protein	Number of patients	Reference
c.235C > T	p.Arg79*	NEMM	1	15
c.1599–56_1621del		HAT	1	13
c.1928A > G	p.Asn643Ser	HAT	1	16
c.3040–1_3040delGA		ACIDIC	1	16
c.3070C > T	p.Arg1024*	ACIDIC	5	3,16,17
c.3116_3117delCT	p.Ser1039*	ACIDIC	2	4
c.3230delA	p.Asn1077Metfs*46	ACIDIC	1	16
c.3287delG	p.Cys1096Phefs*27	ACIDIC	1	16
c.3338C > G	p.Ser1113*	ACIDIC	1	18
c.3385C > T	p.Arg1129*	ACIDIC	3	3
c.3390delA	**p.Lys1130Asnfs*4**	**ACIDIC**	**1**	**Present study**
c.3462delA	p.Gly1155Alafs*21	ACIDIC	1	16
c.3655del	p.Leu1219Tyrfs*	ACIDIC	1	19
c.3830_3831insTT	p.Arg1278Serfs*17	ACIDIC	1	4
c.3850_3851dup	p.Gln1285Glyfs*10	ACIDIC	1	20
c.3879dupA	p.Glu1294Argfs*19	ACIDIC	1	4
c.4108G > T	p.Glu1370*	ACIDIC	1	4
c.4292dupT	p.Leu1431Phefs*8	ACIDIC	1	4
c.4688_4689del	p.Tyr1563*	ACIDIC	1	21
c.5924A > G	p.Asn1975Ser	SER/MET	2	14
Whole gene deletion			1	4

Abbreviations: ACIDIC, acidic glutamate/aspartate-rich domain; HAT, histone acetyltransferase domain; NEMM, nuclear localization domain; SER/MET, serine and methionine-rich transactivation domain.
Note: The one in bold represents the mutation in current study. KAT6A protein domains.

delay. Clinical WES identified a novel mutation in *KAT6A* gene. This mutation is an explanation for the presentation. This is supported by the expected severe impact of the truncating mutation on protein structure and function and recent multiple reports of patients (28 patients, 20 different mutations) associating mutations in this gene with autosomal dominant mental retardation type 32 (OMIM no. 616268) (►**Table 1**).

The *KAT6A* gene encodes a histone acetyltransferase (HAT) that is a part of a multiprotein complex functioning in multiple cellular processes such as maintenance of adult stem cells, differentiation, senescence, and apoptosis.[9,10] *KAT6A* acetylates lysine 9 residue in histone H3 (H3K9). This process is associated with activating transcription, while deacetylation is associated with inhibiting transcription. Functional analysis of histone extracts in fibroblasts from an individual with a mutation in *KAT6A* showed decreased H3K9 acetylation and increased H3K18 acetylation.[3] That patient had the p. Arg1129* mutation, which is very close to our patient's mutation (p.Lys1130Asnfs*4). *KAT6A* is expressed abundantly in the brain during fetal development and disruption of *BRPF1*—a chromatin regulator and activator of *KAT6A*—causes abnormal brain development.[11] *KAT6A* also regulates *TBX1*, with mutations of the latter causing DiGeorge syndrome. Knockout of *KAT6A* in mice was found to phenocopy DiGeorge syndrome with severe abnormalities affecting the brain.[12] Containing 18 exons, *KAT6A*, encodes a 2004 amino acid protein with five major domains including nuclear localization domain (NEMM), double-plant zinc finger homeodomain (PHD) which binds to acetylated histone tails, HAT domain, acidic glutamate/aspartate-rich domain (ACIDIC), and serine and methionine-rich transactivation domain (SER/MET). The mutation reported in our patient is expected to disrupt the normal acetylation of many genes including p53,[3] and consequently dysregulate downstream signaling which may affect different cellular functions such as apoptosis and organogenesis through dysregulated chromatin modification.

The majority of *KAT6A* mutations reported cluster in exons 17 and 18 within the ACIDIC domain indicating its importance to the proper functioning of the protein. A literature review of publications describing patients and mutations in *KAT6A* identified 29 patients and 21 different mutations (including our patient); nine mutations are frameshift, seven truncating, two deletions, two missense, and one whole gene deletion (►**Table 1**). Interestingly, deletion of the whole gene caused only a mild intellectual disability phenotype with the patient being able to attend mainstream schooling.[4] In addition, a ~70-bp deletion (including 21bp of coding sequence) only yielded a speech phenotype without global developmental delay.[13] Furthermore, a missense mutation toward the end of the protein (at position 1975 from the 2004 amino acid protein) was reportedly inherited from father to daughter. Both father and daughter had mild phenotypic features, with the father showing a milder phenotype than his daughter and thus displaying intrafamilial variability.[14]

Common features shared by our patient and previously reported patients include global developmental delay, intellectual disability, delayed motor skills, delayed speech, neutropenia, neonatal hypotonia, and feeding difficulties. Some features reported in other patients that are not present in our patient include microcephaly, short stature, facial dysmorphism, nasal anomalies, cleft palate, dental anomalies, strabismus, epicanthal folds, ptosis and cardiac, and pulmonary defects. Furthermore, many patients with *KAT6A* mutations underwent MRI but major brain abnormalities are rare. Mild abnormalities such as thin corpus callosum or delayed myelination have been reported,[4,15,16] most of which resolved over time. In our patient, the second MRI showed improved features, consistent with previous reports.

In conclusion, we report a patient with a novel mutation in *KAT6A*. Since 2015 multiple reports have described patients with *KAT6A* mutations. This report seeks to expand our clinical knowledge of the phenotypic features caused by a different and novel mutation affecting the same gene. Furthermore, it supports the importance of WES to detect the genetic cause for undiagnosed patients with nonspecific phenotypes.

Conflict of Interest
None declared.

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